

# An experimental study of carbon-isotope fractionation between diet, hair, and feces of mammalian herbivores

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**Abstract:** The carbon-isotope composition of hair and feces offers a glimpse into the diets of mammalian herbivores. It is particularly useful for determining the relative consumption of browse and graze in tropical environments, as these foods have strongly divergent carbon-isotope compositions. Fecal  $\delta^{13}\text{C}$  values reflect the last few days consumption, whereas hair provides longer term dietary information. Previous studies have shown, however, that some fractionation occurs between dietary  $\delta^{13}\text{C}$  values and those of hair and feces. Accurate dietary reconstruction requires an understanding of these fractionations, but few controlled-feeding studies have been undertaken to investigate these fractionations in any mammalian taxa, fewer still in large mammalian herbivores. Here, we present data from the first study of carbon-isotope fractionation between diet, hair, and feces in multiple herbivore taxa. All taxa were fed pure alfalfa (*Medicago sativa*) diets for a minimum period of 6 months, at which point recently grown hair was shaved and analyzed for carbon isotopes. The mean observed diet–hair fractionation was +3.2‰, with a range of +2.7 to +3.5‰. We also examined diet–feces fractionation for herbivores on alfalfa and bermudagrass (*Cynodon dactylon*) feeds. The mean diet–feces fractionation for both diets was –0.8‰, with less fractionation for alfalfa (–0.6‰) than bermudagrass (–1.0‰). Fecal carbon turnover also varies greatly between taxa. When diets were switched, horse (*Equus caballus*) feces reflected the new diet within 60 h, but alpaca (*Lama pacos*) feces did not equilibrate with the new diet for nearly 200 h. Thus, fecal carbon isotopes provide far greater dietary resolution for hindgut-fermenting horses than foregut-fermenting alpacas.

**Résumé :** La composition en isotopes de carbone du poil et des fèces donne un aperçu du régime alimentaire des mammifères herbivores. Elle sert, entre autres, à déterminer la consommation relative de brout et d'herbes dans les milieux tropicaux, puisque ces nourritures ont des compositions très différentes en isotopes de carbone. Les valeurs de  $\delta^{13}\text{C}$  des fèces reflètent la composition du régime alimentaire au cours des quelques derniers jours précédant l'analyse, alors que celles du poil sont des indicateurs du régime à plus long terme. Des études antérieures ont cependant révélé qu'il se produisait un fractionnement entre les valeurs de  $\delta^{13}\text{C}$  mesurées directement dans le régime alimentaire et celles des fèces et des poils. La reconstruction exacte du régime alimentaire suppose une bonne compréhension préalable de ces fractionnements, mais peu d'études sur le régime alimentaire dans des conditions contrôlées ont été entreprises pour évaluer ces fractionnements chez les taxons de mammifères, encore moins chez les mammifères herbivores de grande taille. Nous présentons ici les données de la première étude du fractionnement des isotopes du carbone entre les aliments, le poil et les fèces chez plusieurs mammifères herbivores. Tous les mammifères ont été soumis à un régime de luzerne (*Medicago sativa*) pendant une période d'au moins 6 mois, après lesquels leur poil récemment poussé a été rasé et analysé pour y évaluer les isotopes de carbone. Le fractionnement des isotopes entre les aliments et le poil était de +3,2 ‰, avec une étendue de +2,7 ‰ à +3,5 ‰. Nous avons également évalué le fractionnement entre les aliments et les fèces chez des herbivores gardés à un régime de luzerne et d'herbes marines (*Cynodon dactylon*). Le fractionnement moyen observé entre les aliments et les fèces pour les deux régimes était de –0,8 ‰ et le fractionnement était moins important avec le régime de luzerne (–0,6 ‰) qu'avec le régime d'herbes marines (–1,0 ‰). Le taux de remplacement du carbone fécal variait aussi fortement d'un taxon à l'autre. À la suite d'un changement de régime, il fallait 60 h avant que les changements deviennent apparents dans les fèces chez le cheval (*Equus caballus*), mais près de

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200 h chez l'alpaga (*Lama pacos*). Conséquemment, la composition en isotopes stables fournit une meilleure résolution du régime alimentaire du cheval, chez lequel la fermentation se fait dans la partie postérieure du tube digestif, que de celui de l'alpaga, chez qui elle se fait dans la partie antérieure.

[Traduit par la Rédaction]

## Introduction

Analysis of stable carbon isotopes has become a common tool for studying the nutritional ecology of wildlife. It has been employed to study diverse taxa such as antelopes (Antilopinae; Tieszen and Imbaba 1980), elephants (*Loxodonta africana*; van der Merwe et al. 1988), raptors (Harding and Stevens 2001), bears (*Ursus americanus* and *Ursus arctos*; Hobson et al. 2000), bandicoots (*Peroryctes* spp.; McIlwee and Johnson 1998), long-finned pilot whales (*Globicephala melas*; Abend and Smith 1997), and chimpanzees (*Pan troglodytes*; Schoeninger et al. 1999). Stable carbon isotope analysis is particularly useful for investigating the diets of mammalian herbivores in tropical environments, as available browse (trees, shrubs, forbs) and graze (monocots) have highly distinct, nonoverlapping carbon-isotope compositions (Smith and Epstein 1971; Deines 1980). Although many materials can be used for stable-isotope analysis, hair and feces are particularly valuable, as they are fairly easy to obtain in field situations from live animals (e.g., Tieszen and Imbaba 1980; Schoeninger et al. 1999). Furthermore, hair can often be collected from rare and difficult to observe nocturnal species without causing unnecessary stress (Schoeninger et al. 1998).

Hair and feces provide different kinds of dietary information. Feces  $\delta^{13}\text{C}$  values reflect an animal's consumption over a relatively short period of time, enabling researchers to investigate short-term dietary fluctuations. The down-side of this is that fecal collection must continue over an extended period if one is to accurately characterize an animal's long-term diet. Bulk hair samples, in contrast, integrate diet over a longer period of time, making it easier to classify species into general dietary types (e.g., grazers, browsers, mixed-feeders). Hence, both materials have advantages and disadvantages, with their relative utility being determined by the nature of the research question.

Nonetheless, our ability to use these materials (particularly hair) to study large mammalian herbivores is hampered because the fractionation of diet to hair, and to a lesser extent, diet to feces is poorly known. Although no experimental studies of large herbivores have been undertaken (but see Jones et al. 1981), controlled and semicontrolled studies of rodents, foxes (*Vulpes* spp.), and seals (*Phoca* spp.) suggest a diet-hair fractionation of +0.7 to +3.2‰ (DeNiro and Epstein 1978; Tieszen et al. 1983; Nakagawa et al. 1985; Roth and Hobson 2000). There is also evidence that hair  $\delta^{13}\text{C}$  values reflect the carbon-isotope composition of dietary protein, rather than the composition of the whole diet (Tieszen and Fagre 1993). This phenomenon is not likely to engender interpretative complications for herbivores, as their dietary protein  $\delta^{13}\text{C}$  values are very similar to those of their whole diets (Fagre et al. 1991; Tieszen 1991).

In contrast, the relationship between dietary and fecal  $\delta^{13}\text{C}$  values in large mammalian herbivores has been investigated in controlled-feeding studies (Jones et al. 1979, 1981). These

experiments suggested that when ruminants are fed pure hay diets, their feces can be enriched in  $^{13}\text{C}$  by up to +2.4‰ or depleted by as much as -2.0‰. Fecal carbon turnover has also been explored (Jones et al. 1979; Coates et al. 1991). Jones et al. (1979) found that when steers were switched from a  $\text{C}_4$  to a  $\text{C}_3$  diet, feces did not fully reflect the new dietary  $\delta^{13}\text{C}$  values for 6 days. In addition, when hindgut-fermenting rabbits (*Oryctolagus cuniculus*) were switched from a  $\text{C}_3$  to a  $\text{C}_4$  diet, fecal  $\delta^{13}\text{C}$  values did not equilibrate with the new diet for 14 days, despite fast passage rates of digesta (Uden et al. 1982). This attenuation of the new dietary signal was probably the result of extensive caecotrophy and selective retention of  $\text{C}_3$  particles in the caecum. It is likely that fecal carbon turnover is much faster in non-caecotrophic hindgut fermenters such as horses (*Equus caballus*), but this possibility has not been explored. Such data on fecal turnover rates in a wide variety of taxa are necessary to maximize the dietary information provided by feces collected in the wild.

Here, we report the results of three controlled-feeding experiments, one on herbivore hair and two on herbivore feces. The hair experiment investigated herbivore diet-hair fractionation on isotopically homogenous alfalfa (*Medicago sativa*) diets (Experiment 1). The first feces experiment explored diet-feces fractionation on alfalfa and bermudagrass (*Cynodon dactylon*) diets (Experiment 2), and the other investigated fecal carbon turnover in pseudoruminant alpacas (*Lama pacos*) and nonruminant horses (Experiment 3).

## Methods

In Experiment 1, we fed eight cattle (*Bos taurus*), four llamas (*Lama glama*), two alpacas, four goats (*Capra hircus*), and four rabbits an alfalfa diet (crude protein = 19%) for 6 months or more. This interval was shown previously to be sufficient for carbon-isotope equilibration between diet and hair (Jones et al. 1981). Each species was housed in a separate pen, and feed and water were available ad libitum. The alfalfa hay was obtained from a single cutting of one farm to minimize isotopic heterogeneity. Hay samples were collected and analyzed for stable isotopes weekly. The  $\delta^{13}\text{C}$  value for the alfalfa hay was  $-27.0 \pm 0.4\text{‰}$ . At the end of the experiment, a patch of hair was clipped to within 1 mm of the skin, and the last millimetre (which represented the most recent growth) was removed with a disposable razor for stable-isotope analysis.

For Experiment 2, we fed four cattle, goats, llamas, alpacas, and horses pure alfalfa and pure bermudagrass diets for 3 weeks. Rabbits were fed alfalfa but not bermudagrass, as they lose mass quickly on the latter. Feed and fecal samples were collected during the final week of the experiment.

In Experiment 3, two foregut-fermenting alpacas and two hindgut-fermenting horses were placed on a brome grass (*Bromus inermis*) diet with a  $\delta^{13}\text{C}$  value of  $-27.0 \pm 0.5\text{‰}$ .

**Table 1.** Sample sizes ( $n$ ),  $\delta^{13}\text{C}$  values, diet–hair fractionations ( $\epsilon^*$ ), and standard deviations (SD) for feed and hair in Experiment 1.

|   | $n$ | $\delta^{13}\text{C}$ | $\epsilon^*$ | SD  |
|---|-----|-----------------------|--------------|-----|
| Diet                                    |     |                       |              |     |
| Alfalfa ( <i>Medicago sativa</i> )      | 17  | -27.0                 | na           | 0.4 |
| Hair                                    |     |                       |              |     |
| Cattle ( <i>Bos taurus</i> )            | 8   | -24.4                 | 2.7          | 0.4 |
| Goat ( <i>Capra hircus</i> )            | 4   | -23.8                 | 3.2          | 0.1 |
| Alpaca ( <i>Lama pacos</i> )            | 2   | -23.9                 | 3.2          | 0.1 |
| Llama ( <i>Lama glama</i> )             | 4   | -23.6                 | 3.5          | 0.2 |
| Rabbit ( <i>Oryctolagus cuniculus</i> ) | 4   | -23.7                 | 3.4          | 0.3 |
| Mean                                    |     | -23.9                 | 3.2          | 0.2 |

Note: na, not applicable.

After 7 weeks, they were switched to an isonitrogenous bermudagrass diet with a  $\delta^{13}\text{C}$  value of  $-13.3 \pm 0.4\text{‰}$ . After the switch, feces were collected every 2 h on the first day, every 4 h on the second day, every 8 h on the third day, and every 12 h for the next 11 days. All fecal samples were dried in a forced-air oven at  $45^\circ\text{C}$  and ground to a particle size of  $<1$  mm.

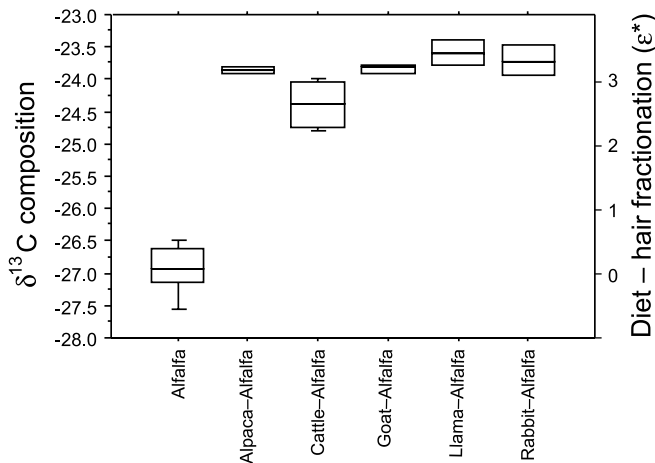
Hair and fecal samples were combusted in an automated Carlo-Erba device (Carlo-Erba, Milan, Italy) and stable carbon isotopes were analyzed using a flow-through inlet system on a continuous-flow isotope-ratio mass spectrometer (Finnigan, Bremen, Germany). The standard deviation for replicate measurements of a yeast standard was  $<0.1\text{‰}$ . Fractionation values are usually expressed in  $\Delta$  notation ( $\delta^{13}\text{C}_{\text{hair/feces}} - \delta^{13}\text{C}_{\text{diet}}$ ; e.g., Ambrose and Norr 1993; Hobson et al. 1996; Ostrom et al. 1997; Webb et al. 1998; Roth and Hobson 2000; Jenkins et al. 2001). Although this is a convenient short-hand for discussing fractionations, it is not strictly correct. Ideally, fractionations should be expressed as  $\epsilon^*$  values,  $[(1000 + \delta^{13}\text{C}_{\text{hair/feces}})/(1000 + \delta^{13}\text{C}_{\text{diet}}) - 1] \times 1000$ , which are sometimes but not always equivalent to  $\Delta$  values (see Cerling and Harris 1999). We follow this convention here.

## Results

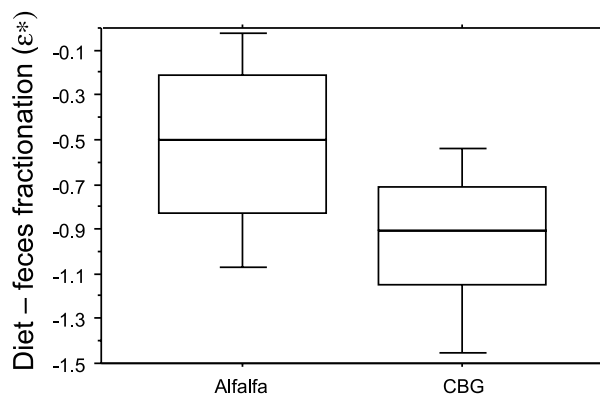
Hair  $\delta^{13}\text{C}$  values are enriched by slightly over  $+3.0\text{‰}$  for all taxa on the high-quality alfalfa diet, except the cattle which are enriched by  $+2.7\text{‰}$  (Table 1, Fig. 1). The mean diet–hair fractionation for all species eating alfalfa is  $+3.2\text{‰}$ . We cannot be certain why the cattle are less enriched in  $^{13}\text{C}$  than the other herbivores, although the cattle were rapidly growing yearlings, whereas the other animals were adults. Thus, the cattle had higher protein requirements and likely used a higher proportion of dietary protein than the other taxa, for which the alfalfa greatly exceeded their protein requirements (Cheeke 1999).

Feces for all taxa on both diets are depleted in  $^{13}\text{C}$  compared with diet by an average of  $-0.8\text{‰}$  (Table 2, Fig 2). Analysis of variance shows that alfalfa ( $-0.6\text{‰}$ ) is fractionated less than coastal bermudagrass ( $-1.0\text{‰}$ ) (ANOVA,  $F_{[1,42]} = 15.908$ ,  $P = 0.0002$ ; Fig. 2). There is no significant difference between the fecal  $\delta^{13}\text{C}$  values of hindgut and foregut fermenters, but there are some interspecific differences. For instance, rabbit feces are more enriched in  $^{13}\text{C}$  than cattle feces when both are consuming alfalfa (Scheffé's test,  $P < 0.05$ ). None-

**Fig. 1.** Boxplot showing  $\delta^{13}\text{C}$  and diet–hair fractionation ( $\epsilon^*$ ) for herbivores and their feed in Experiment 1. Diet–hair fractionation ranges from  $+2.7\text{‰}$  for cattle to  $+3.5\text{‰}$  for llamas.



**Fig. 2.** Boxplot showing diet–feces fractionation ( $\epsilon^*$ ) for herbivores on alfalfa and coastal bermudagrass (*Cynodon dactylon*, CBG) hays.



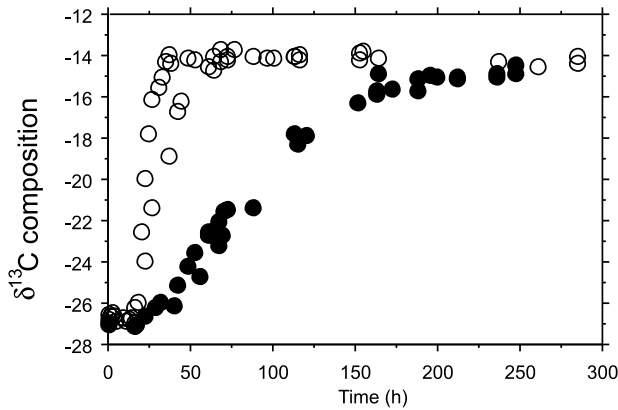
theless, these interspecific differences are never greater than  $0.7\text{‰}$  and can probably be ignored when reconstructing diets from fecal stable-isotope data.

As expected, fecal carbon turnover was much faster in hindgut-fermenting horses than foregut-fermenting alpacas (Fig. 3). After a switch from a  $\text{C}_3$  to a  $\text{C}_4$  grass hay, horse fecal and dietary  $\delta^{13}\text{C}$  values equilibrated within 60 h, whereas alpaca feces did not fully reflect the new diets for nearly 200 h.

## Discussion

This study presents the first experimental evidence of diet–hair fractionation in multiple large herbivore taxa. The mean fractionation value of  $+3.2\text{‰}$  fits well with previous estimates of diet–hair fractionation in herbivores (Cerling and Harris 1999) and results from controlled-feeding studies of terrestrial carnivores (Roth and Hobson 2000) and marine mammals (Hobson et al. 1996). Studies of arthropods suggest that dietary quality affects diet–tissue fractionation (Webb et al. 1998; Oelbermann and Scheu 2002), but it remains to be seen if dietary quality affects carbon-isotope fractionation in mammals. If so, this could have important implications be-

**Fig. 3.** Scattergram showing fecal  $\delta^{13}\text{C}$  values for two alpacas (●) and two horses (*Equus caballus*, ○) that have been switched from a  $\text{C}_3$  to a  $\text{C}_4$  diet. The carbon isotope composition of horse feces reflects the new diet within 60 h, but alpaca feces does not fully reflect the new diet for almost 200 h.



**Table 2.** Sample sizes ( $n$ ),  $\delta^{13}\text{C}$  values, diet–feces fractionations ( $\epsilon^*$ ), and standard deviations (SD) for feeds and feces in Experiment 2.

|                            | $n$ | $\delta^{13}\text{C}$ | $\epsilon^*$ | SD  |
|----------------------------|-----|-----------------------|--------------|-----|
| <b>Diets</b>               |     |                       |              |     |
| Alfalfa                    | 4   | -27.0                 | na           | 0.2 |
| Coastal bermudagrass (CBG) | 4   | -13.3                 | na           | 0.3 |
| <b>Feces</b>               |     |                       |              |     |
| Alfalfa–alpaca             | 4   | -27.4                 | -0.4         | 0.4 |
| Alfalfa–cattle             | 4   | -28.0                 | -1.0         | 0.2 |
| Alfalfa–goat               | 4   | -27.7                 | -0.8         | 0.1 |
| Alfalfa–llama              | 4   | -27.4                 | -0.4         | 0.5 |
| Alfalfa–rabbit             | 4   | -27.3                 | -0.3         | 0.1 |
| Alfalfa–horse              | 4   | -27.4                 | -0.5         | 0.4 |
| Mean                       |     | -27.5                 | -0.6         | 0.3 |
| CBG–cattle                 | 4   | -14.2                 | -0.9         | 0.2 |
| CBG–goat                   | 4   | -14.3                 | -1.0         | 0.4 |
| CBG–llama                  | 4   | -14.5                 | -1.2         | 0.4 |
| CBG–alpaca                 | 4   | -14.7                 | -1.3         | 0.2 |
| CBG–horse                  | 4   | -14.0                 | -0.7         | 0.2 |
| Mean                       |     | -14.3                 | -1.4         | 0.3 |

**Note:** na, not applicable.

cause fractionation may be different for browsing and grazing herbivores, as the former tend to consume diets higher in crude protein than the latter (Robbins 1993). As secondary compounds can deter protein utilization (Robbins et al. 1987; Hanley et al. 1992), they might also affect diet–hair fractionation. Controlled-feeding studies that test these possibilities by altering dietary quality (e.g., dietary protein, fiber, and secondary compounds) are required.

The mean  $-0.8\text{‰}$  diet–feces fractionation found here is less than has been reported for similar animals on natural forages ( $-1.9\text{‰}$  in Jones et al. 1981). Rodents on pelleted lab diets can also have larger diet–feces fractionations ( $-2.1\text{‰}$  in Nakagawa et al. 1985), although pigs (*Sus scrofa*) on lab diets can have similar fractionations ( $-0.4\text{‰}$  in Hare et al. 1991). The reduced fractionation reported here at least partially reflects the inclusion of alfalfa in this study, which as discussed

**Table 3.** Bulk, acid–detergent fiber (ADF), and acid–detergent solubles (ADS)  $\delta^{13}\text{C}$  values for plants and herbivore feces.

|                           | Bulk  | ADF   | ADS   |
|---------------------------|-------|-------|-------|
| <b>Plants</b>             |       |       |       |
| <i>Bromus inermis</i>     | -31.2 | -29.7 | -31.6 |
| <i>Cynodon dactylon</i>   | -14.1 | -12.7 | -14.5 |
| <i>Lolium multiflorum</i> | -31.5 | -29.5 | -31.8 |
| <i>Pennisetum glaucum</i> | -13.1 | -12.6 | -13.3 |
| <i>Medicago sativa</i>    | -27.0 | -27.1 | -26.9 |
| <b>Herbivore feces</b>    |       |       |       |
| Rabbit                    | -27.4 | -27.6 | -27.2 |
| Horse                     | -27.3 | -27.2 | -27.3 |
| Alpaca                    | -27.4 | -27.6 | -27.4 |
| Llama                     | -27.0 | -27.8 | -26.7 |

**Note:** Bulk and ADF  $\delta^{13}\text{C}$  composition values are means of three analyses. ADS values are mass-balance calculations.

above, is little fractionated compared with grass hay. However, it is still unknown why any negative fractionation should occur. Indeed, this negative fractionation is somewhat counterintuitive. The refractory dietary components that compose a plant's acid–detergent fiber (ADF) are enriched in  $^{13}\text{C}$  (Table 3). As these materials are found in a higher proportion in feces than in diets, one might expect feces to have elevated  $\delta^{13}\text{C}$  values. Hence, the observed diet–feces fractionation runs counter to expectations. One possible explanation for this phenomenon is that the large microfloral component of feces is depleted in  $^{13}\text{C}$ , leading to the negative fractionation. But when microbial remains are removed from feces using acid–detergent,  $\delta^{13}\text{C}$  values do not increase (Table 3). Therefore, we cannot explain the negative diet–feces fractionation at present. Nevertheless, diet–feces fractionation for herbivores consuming natural forages seems small and fairly consistent, and should not significantly compromise our ability to make dietary inferences from fecal  $\delta^{13}\text{C}$  values.

Diet–feces fractionation is more complicated when herbivores eat mixed-diets containing several components with different digestibilities, as the poorly digested component will be over-represented in the feces (Jones et al. 1979). This is often the case with premixed lab diets, which often contain a variety of ingredients with disparate isotopic compositions and digestibilities (e.g., corn, alfalfa, binders). However, differential digestibility is unlikely to significantly bias fecal  $\delta^{13}\text{C}$  values in the field. Studies of the digestibility of tree and shrub leaves have shown that they are generally no more digestible than grasses (Hanley et al. 1992; Robbins et al. 1995). Many wild fruits are also very high in fiber (Hart 1985; Mueller et al. 1998), and probably not much more digestible than browse or graze. Thus, it may be that differential digestibility will not greatly complicate dietary reconstruction from fecal  $\delta^{13}\text{C}$  values.

The other crucial aspect of fecal carbon-isotope studies is determining the period of time represented by any given fecal sample. Our data show that horse feces integrate diet over a period of 3 days, while alpaca feces integrate diet over periods greater than a week. These results are consistent with previous studies of passage rates in these two taxa, which have shown that horses have much faster passage

rates (Uden et al. 1982; San Martin 1987; Sponheimer et al. 2002). One might also expect that, in general, hindgut-fermenter feces will preserve finer scale dietary information than ruminant feces. Nevertheless, both ruminant and nonruminant feces offer higher resolution dietary information than hair, which integrates diet over a period of months (Jones et al. 1981; O'Connell and Hedges 1999).

Hair and feces are dietary archives that are easily acquired and can even be used to investigate the diets of individuals that have been dead for thousands of years (White 1993; Macko et al. 1999). Hair  $\delta^{13}\text{C}$  values, in particular, are useful supplements to traditional observational studies. Long-term observation of even a single population of animals is difficult, making interpopulational comparison exceptionally difficult and often impossible. But as hair records diet without the researcher being present, it is an ideal tissue for interpopulational comparisons. Hair carbon-isotope data may also facilitate the study of nocturnal species, many of which are difficult to observe.

Nonetheless, proper utilization of this tool requires a fuller understanding of the processes that lead to fractionation of dietary carbon. Although controlled-feeding studies of large taxa are often difficult and expensive, they offer our best hope of understanding these fractionations.

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